

Antigen-binding units

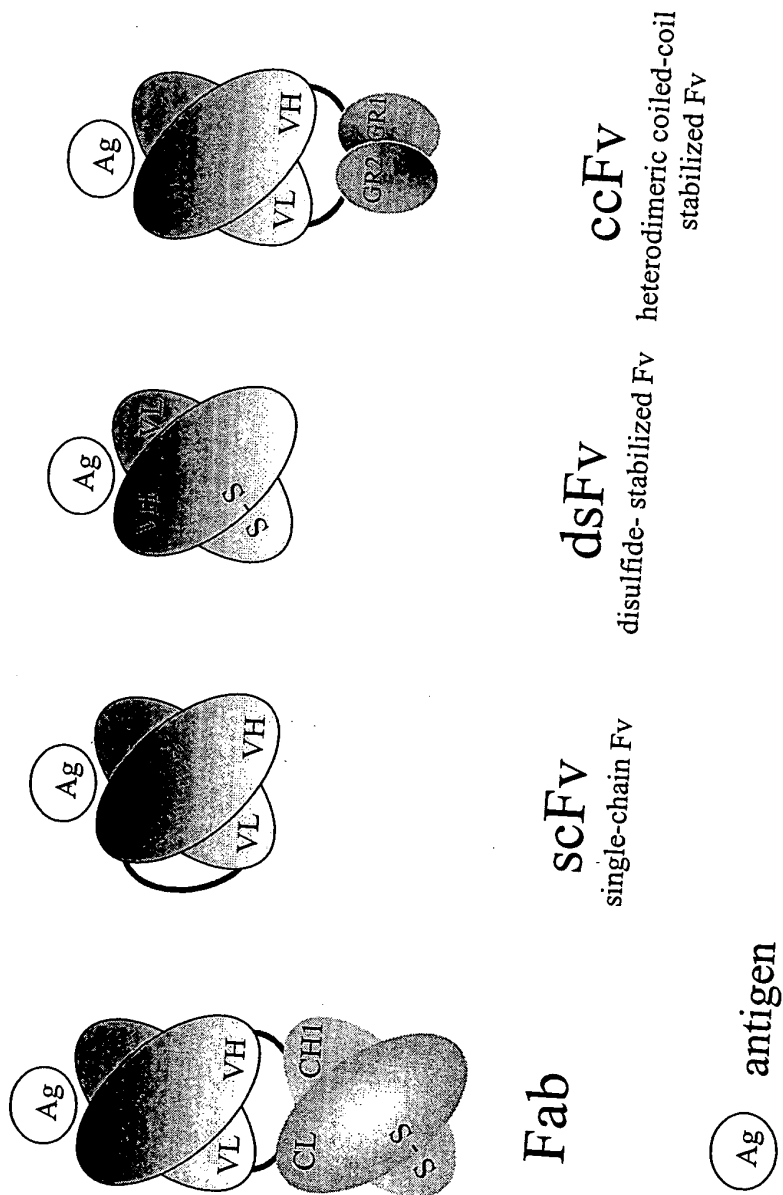


Fig. 1

101080"444T2660

Sequences of coiled-coil domain in ccFv

GR1 Sequence Range: 1 to 146

```
XbaI 10 20 30 40 50
TCTAGAGGTGGAGGAGGTGAGGACATCCCGGCTGTTGGAGAGGAGAA
S R G G G G E E K S R L L E K E N
60 70 80 90 100
CCGTGAACGTGAAAGATCATCTGTGAGAAAGAGGCGTGCTCTGAAC
R E L E K I I A E K E E R V S E
110 120 130 140 AscI
TGCGCCATCAACTCCAGTCTGTAGGAGGTTGTTAATAGGCGCGCC
L R H Q L Q S V G G C * *
```

GR2 Sequence Range: 1 to 140

```
XhoI 10 20 30 40 50
TCTCGAGGAGGTGGTGAACATCCCGCCTGGAGGGCCTACAGTCAGAAA
S R G G G G T S R L E G L Q S E N
60 70 80 90 100
CCATCGCCTGCGAATGAAGATCACAGAGCTGGATAAAGACTTGAAGAGG
H R L R M K I T E L D K D L E E
110 120 130 NotI 140
TCACCATGCAGCTGCAGGACGTCGGAGGTTGCCGGCGCGC
V T M Q L Q D V G G C A A A
```

Fig. 2

Vectors for antibody expression

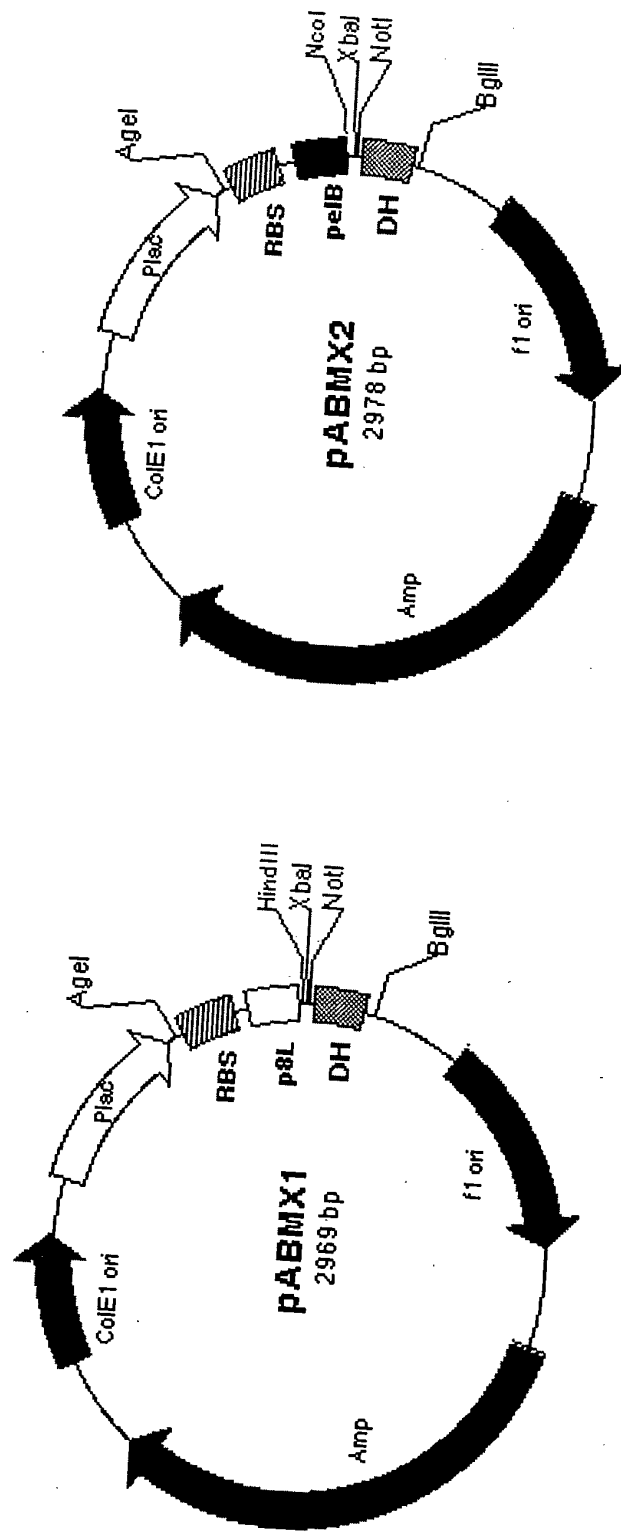


Fig. 3A

PABMX1 vector: sequence from AgeI to BglIII

```

lac promoter/lac O1      AgeI      EP      S/D
AATTGTGAGCGGATAACAATTT ACCGGT TCCT TTAACCTTAG TAAGGAGG AATTAAAAA
P8 Leader
ATGAAAAAGTCTTTAGTCCCTCAAAGCCTCCGTAGCCGTTGCTACCCCTCGTTCCGATGCTAAGCTTCGCT TCTAGA
M K K S L V L K A S V A V A T L V P M L S F A S R
NotI      HA-tag      His-tag      BglIII
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT
A A A Y P Y D V P D Y A G G H H H H H * R S

```

PABMX2 vector: sequence from AgeI to BglIII

```

lac promoter/lac O1      AgeI      EP      S/D
AATTGTGAGCGGATAACAATTT ACCGGT TCCT TTAACCTTAG TAAGGAGG AATTAAAAA
pelB Leader
ATGAAATACCTATTGCCCTACGGCAGCCGCTGGATTGTTATTACTCGGGCCCCAGCCGGCCATGGCGGCCCTGCAGGCCCTCTAGA
M K Y L L P T A A A G L L L L A A Q P A M A A L Q A S R
NotI      HA-tag      His-tag      BglIII
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT
A A A Y P Y D V P D Y A G G H H H H H * R S

```

Fig. 3B

Vectors for antibody display

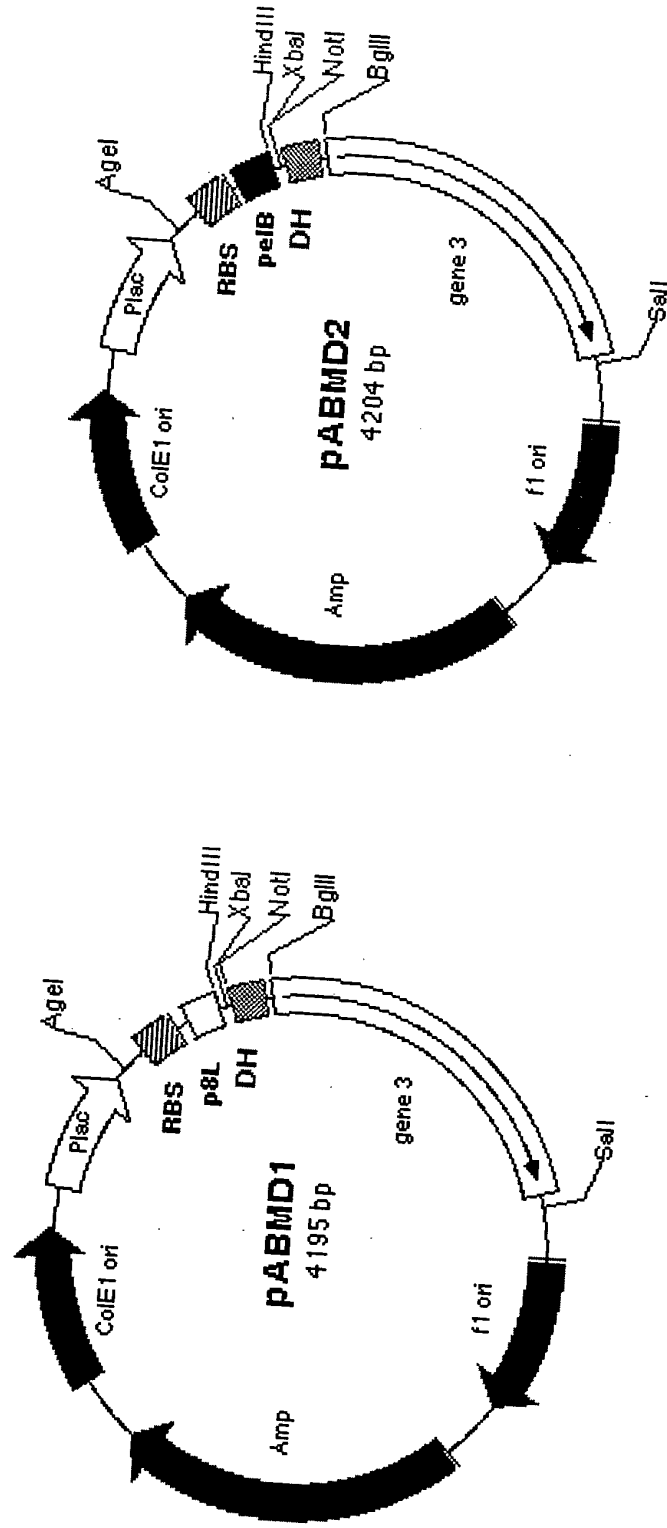


Fig. 4A

101080" 44712560

PABMD1 vector: sequence from AgeI to SalI

```

lac promoter/lac O1      AgeI      EP      S/D
AATTGTGAGCGGATAACAATT ACCGGT TCCT TTAACCTTTAG TAAGGAGG AATTAAAAA
P8 Leader
ATGAAAAAGTCTTTAGTCCCTCAAAGCCTCCGTAGCCGTTGCTACCCCTCGTTCGATGCTAAGCTTCGCT TCTAGA XbaI
M K K S L V L K A S V A V A T L V P M L S F A S R
NotI
CGGGCCGCT TATCCATACGACGTACCGAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT BglII
A A A Y P Y D V P D Y A G G H H H H H * R S
Gene 3
GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA ---- GCTAACATACCTGCGTAATAAGGAGTCTTAA GTCGAC SalI
G G G T V E S C L A K ---- A N I L R N K E S *

```

PABMD2 vector: sequence from AgeI to SalI

```

lac promoter/lac O1      AgeI      EP      S/D
AATTGTGAGCGGATAACAATT ACCGGT TCCT TTAACCTTTAG TAAGGAGG AATTAAAAA
pelB Leader
ATGAAATACCTATTGCTACGGCAGCCGCTGGATTGTTATTACTCGGGCCAGCCGGCCCATGGCGCCCTGCAGGCCCTCTAGA XbaI
M K Y L L P T A A A G L L L A A Q P A M A A L Q A S R
NotI
CGGGCCGCT TATCCATACGACGTACCGAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT BglII
A A A Y P Y D V P D Y A G G H H H H H * R S
Gene 3
GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA ---- GCTAACATACCTGCGTAATAAGGAGTCTTAA GTCGAC SalI
G G G T V E S C L A K ---- A N I L R N K E S *

```

Fig. 4B

Vectors for ccFv expression

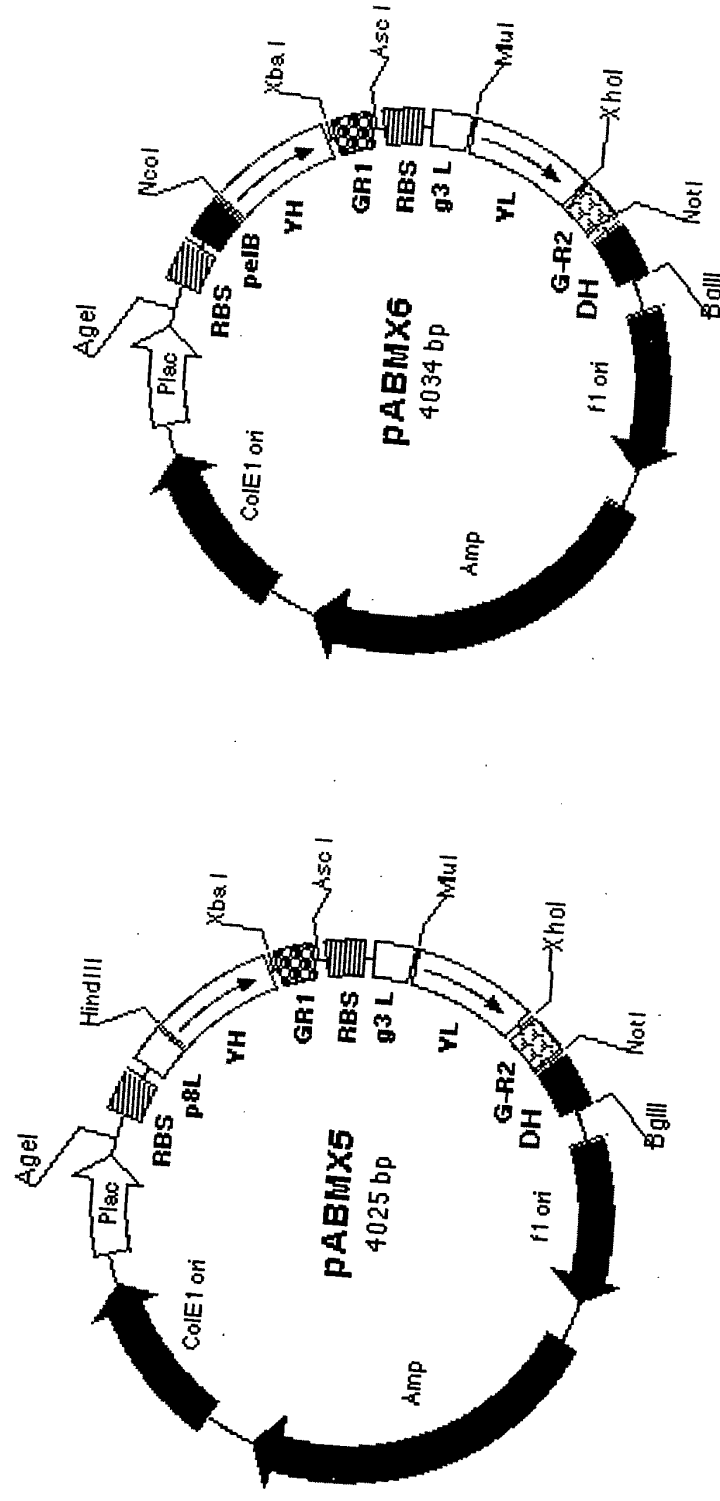


Fig. 5A

Vectors for ccFv display

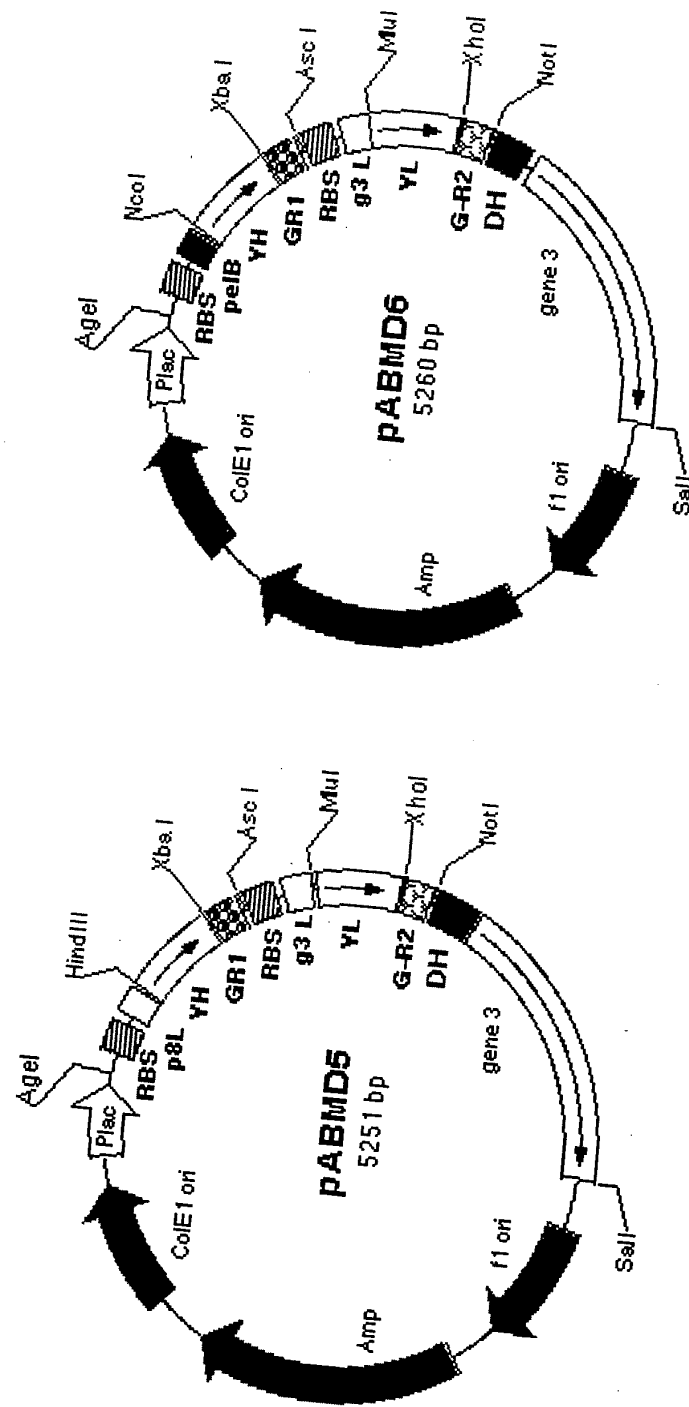


Fig. 6A

101030-4472660

PABMD5 vector: sequence from HindIII to SalI

P8 Leader
 ATGAAAAAGTCTTTAGTCCCTCAAAAGCCTCGTAGCCGTTGCTACCCCTCGTTCCGATGCTAAAGCTTCGCT VH XbaI
TCTAGA
 M K K S L V L K A S V A V A T L V P M L S F A S R

GR1 Ascl
 GGCGCGCCCAACAATTTACACAGTAAGGAGTTTAACTT ATGAAAAAATTATTATTCGCAATTCTTTAGTTGTTCTT
 M K K L L F A I P L V P

S/D
 P3 Leader
 HA-tag
 NotI
 XhoI
 MluI
 TTCTATTCTCACTCCGCTACGCGT VL GR2 TCTCGA S R
 F Y S H S A T R A A A Y P Y D V P D Y A

His-tag
 GGAGGT CATCACCATCATCACCAT TAG GGAGGCGGT ACTGTTGAAAGTTGT---CTGCGTAATAAGGAGTCTTAA SalI
GTCGAC
 G G H H H H H * G G G T V E S C --- L R N K E S *

PABMX6 vector: sequence from pelB leader to DH-tag

pelB Leader
 ATGAAAATACCTATTGCTTACGGCAGCCGCTGGATTGTTATTACTCGCGCCCGCCGCGCATGGCG VH XbaI
TCTAGA
 M K Y L L P T A A A G L L L L A A Q P A M A S R

GR1 Ascl
 GGCGCGCCCAACAATTTACACAGTAAGGAGTTTAACTT ATGAAAAAATTATTATTCGCAATTCTTTAGTTGTTCTT
 M K K L L F A I P L V P

S/D
 P3 Leader
 HA-tag
 NotI
 XhoI
 MluI
 TTCTATTCTCACTCCGCTACGCGT VL GR2 TCTCGA S R
 F Y S H S A T R A A A Y P Y D V P D Y A

His-tag
 GGAGGT CATCACCATCATCACCAT TAG GGAGGCGGT ACTGTTGAAAGTTGT---CTGCGTAATAAGGAGTCTTAA SalI
GTCGAC
 G G H H H H H * G G G T V E S C --- L R N K E S *

Fig. 6B

Vector for ccFv expression in Yeast

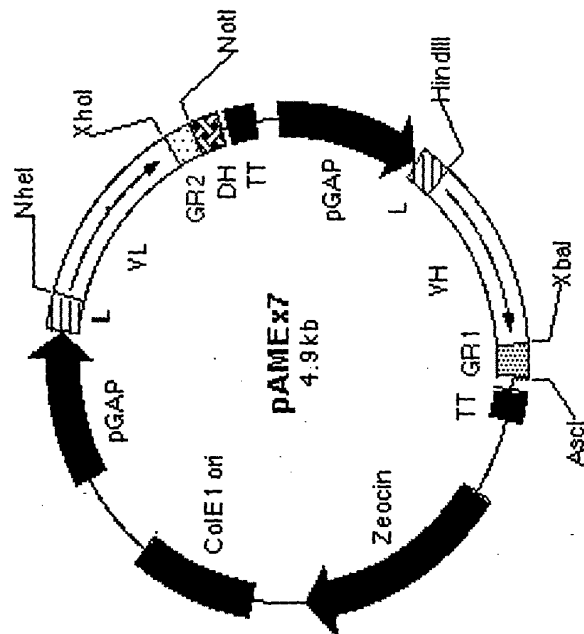


Fig. 7

Soluble antibody ELISA

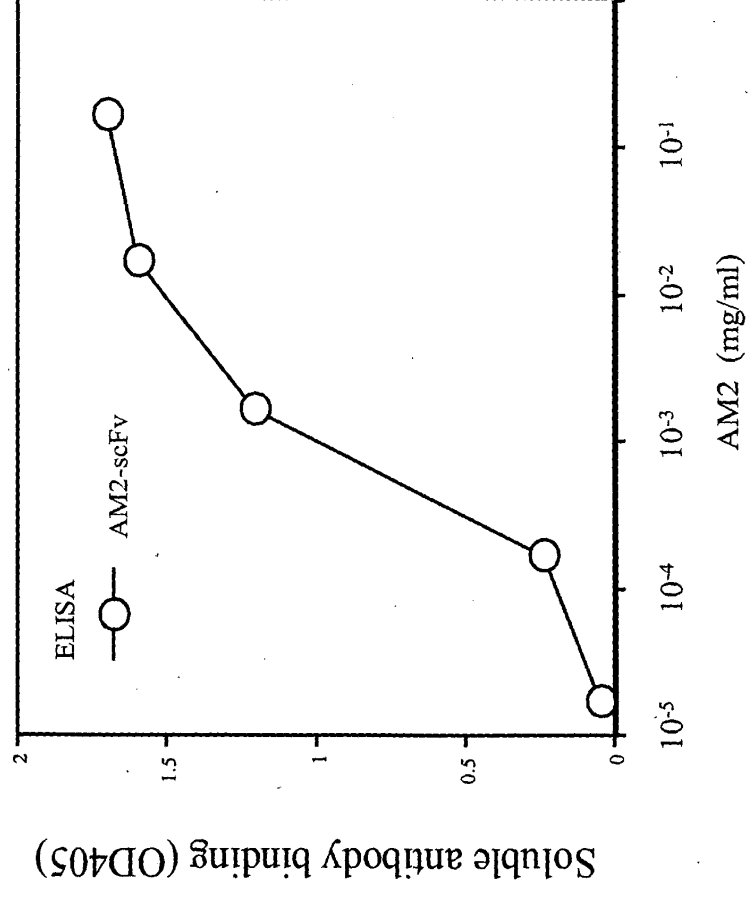


Fig. 8

AM2-scFv display on phage by pABMD1 vector

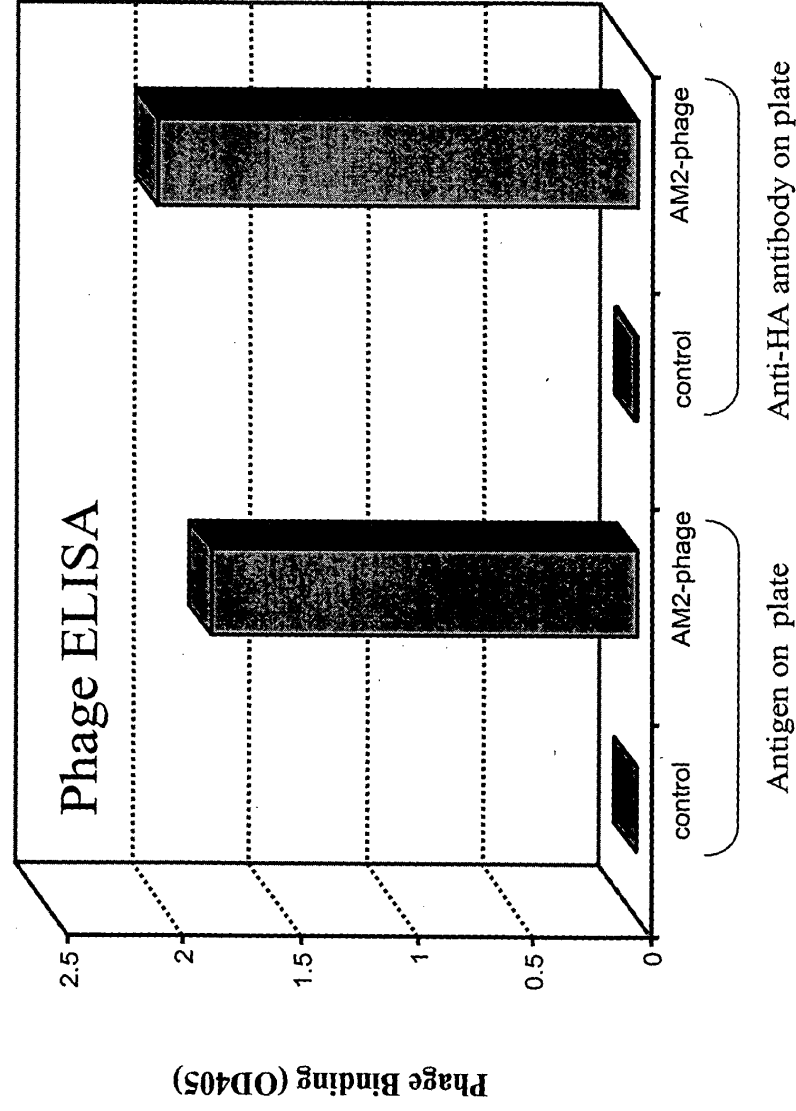


Fig. 9

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Soluble AM1-ccFv antibody expression in E. coli

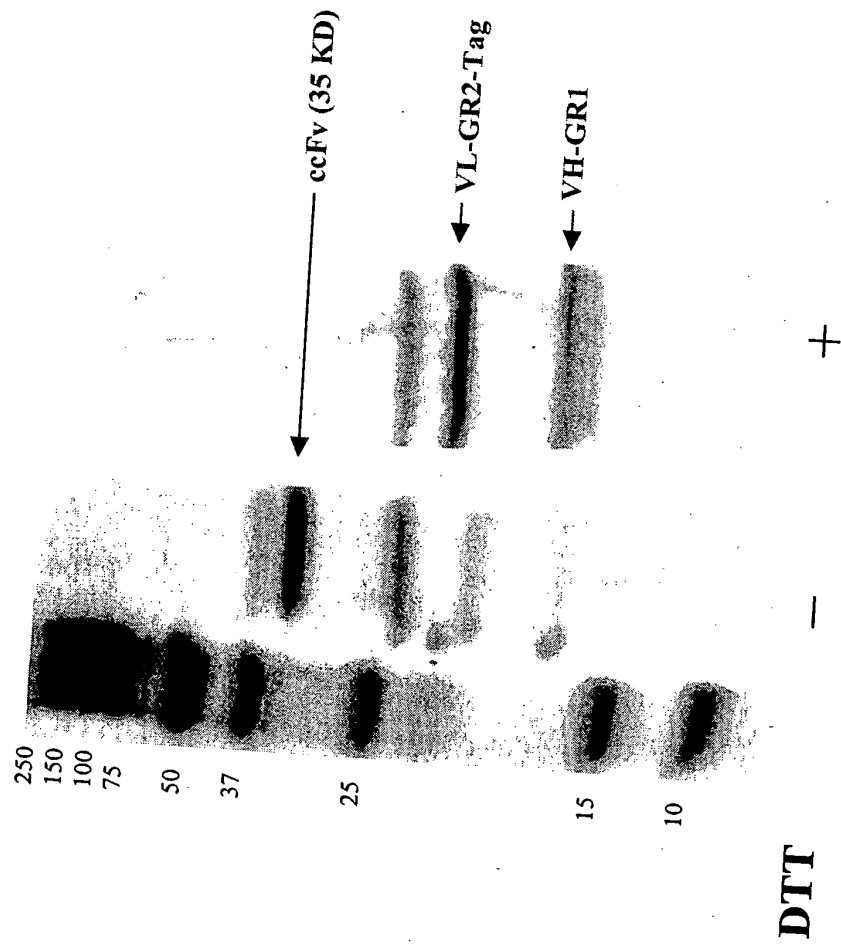


Fig. 10A

Soluble AM1-ccFv antibody binds to antigen

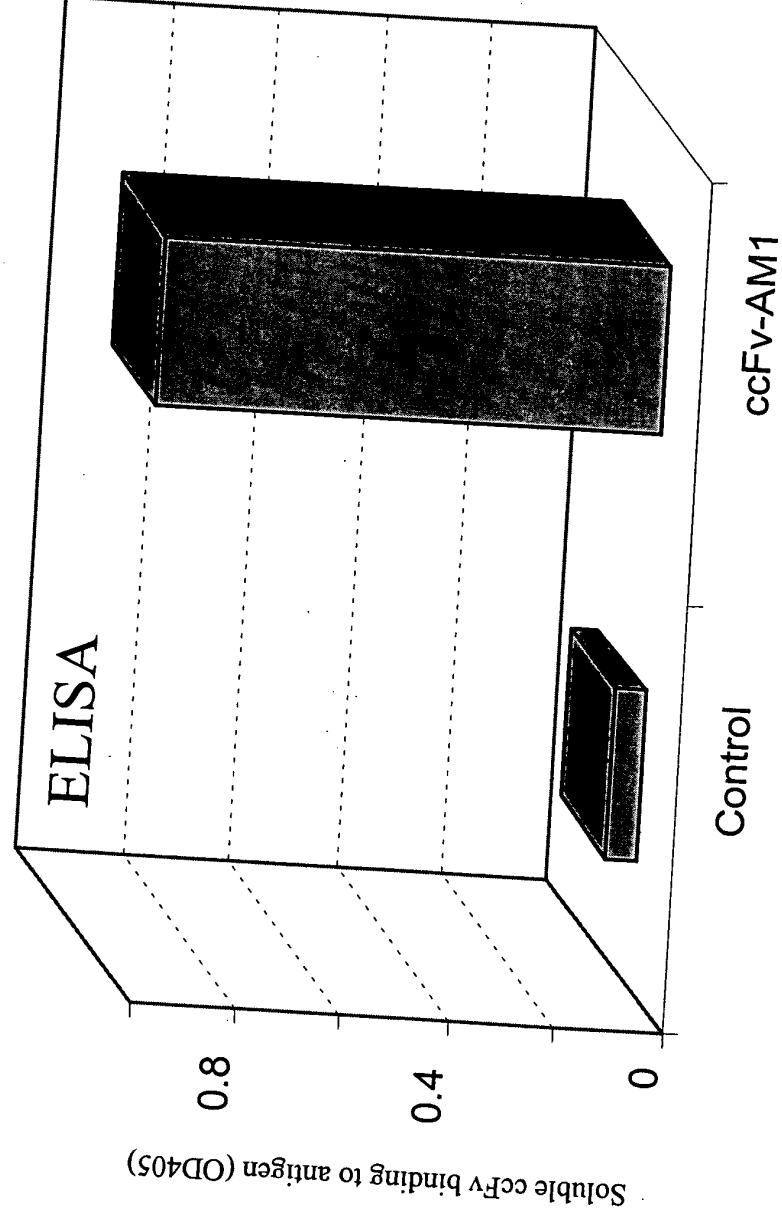


Fig. 10B

AM1-ccFv Antibody display on phage

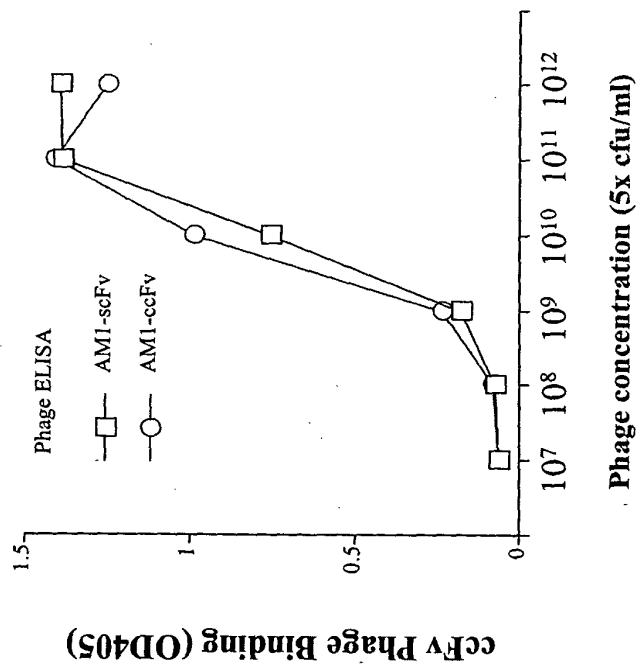


Fig. 11A

Comparison of antigen binding capability of AM2-ccFv and AM2-scFv displayed on phage particles

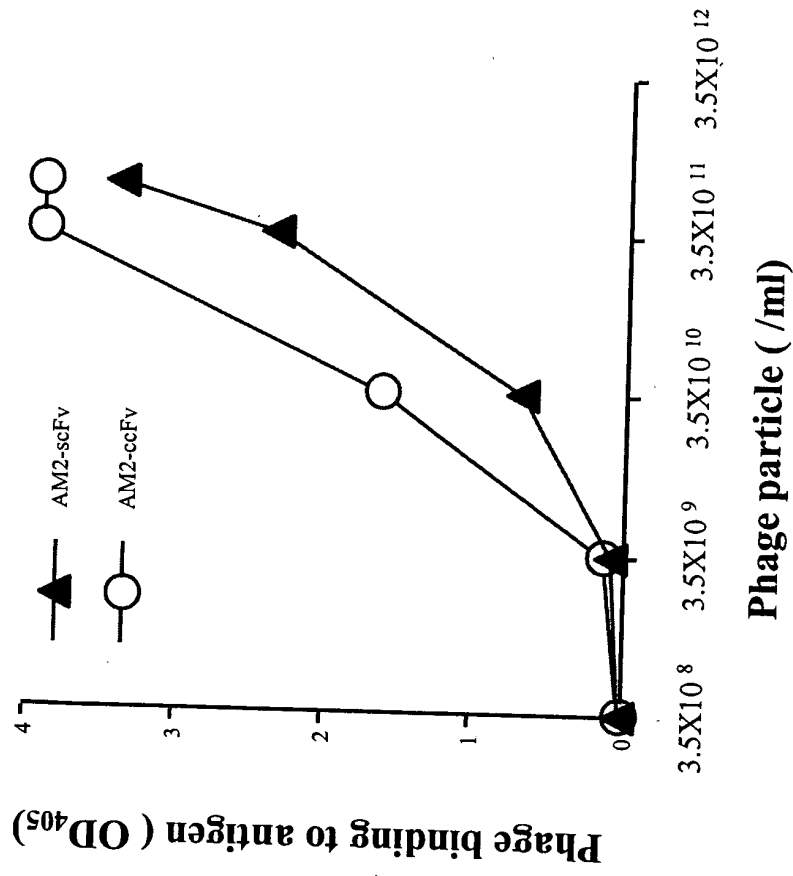


Fig. 11B

Multi-valent ccFv antibody

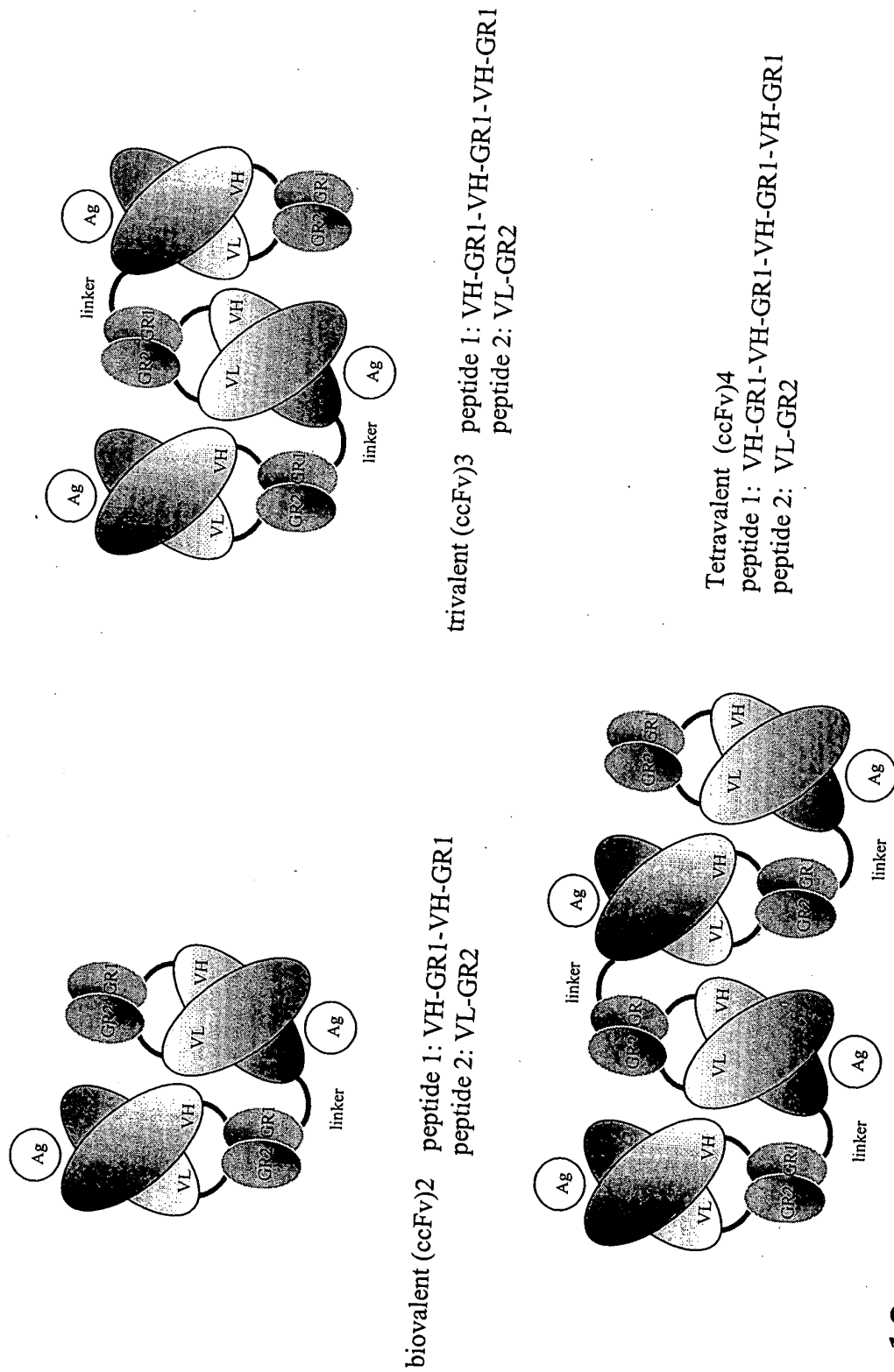
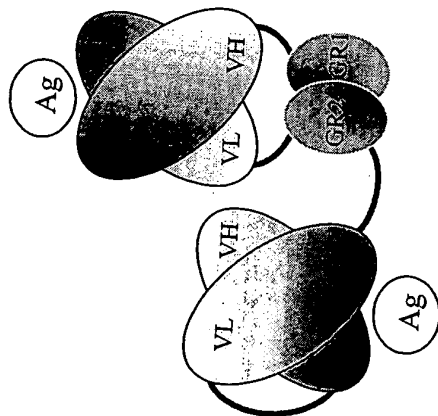
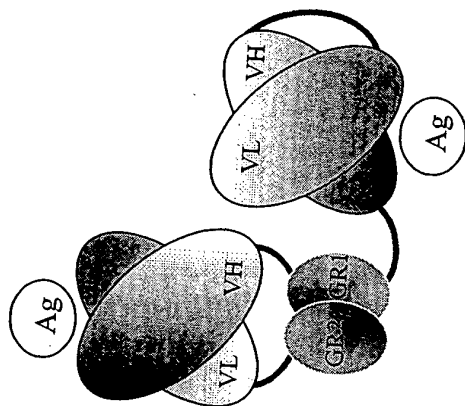


Fig. 12

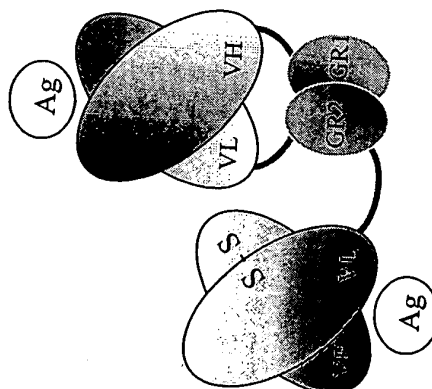
Bi-valent ccFv-scFv/dsFv antibody



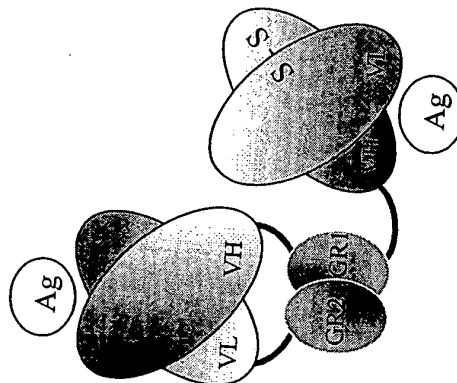
ccFv-scFv
Peptide 1: VL-GR2-scFv
Peptide 2: VH-GR1



ccFv-scFv
Peptide 1: VH-GR1-scFv
Peptide 2: VL-GR2



ccFv-dsFv
Peptide 1: VL-GR2-VL-s
Peptide 2: VH-GR1
Peptide 3: VH-s

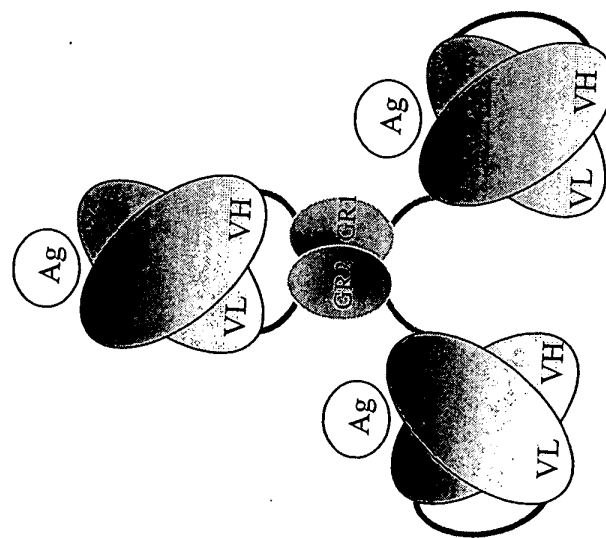


ccFv-dsFv
Peptide 1: VH-GR1-VH-s
Peptide 2: VL-GR2
Peptide 3: VL-s

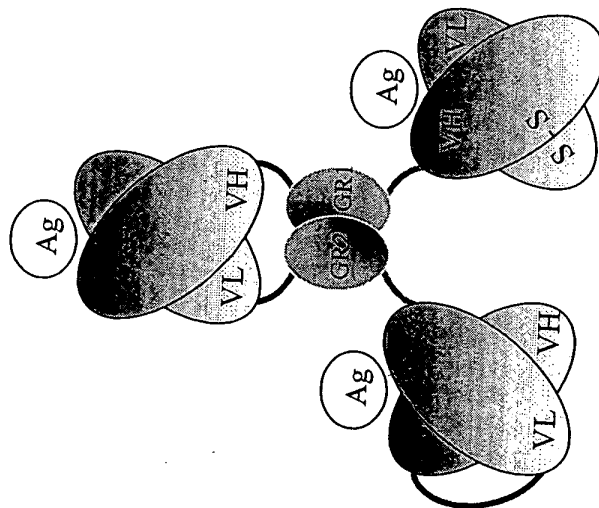
ccFv-dsFv

Fig. 13

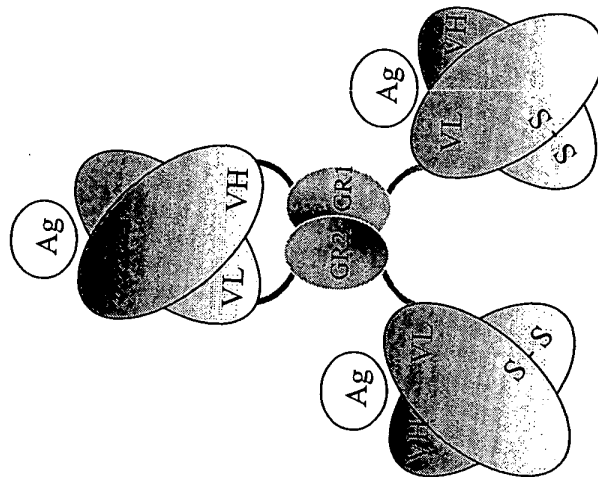
Tri-valent ccFv-scFv/dsFv antibody



Tri-valent ccFv-(scFv)2
 Peptide 1: VH-GR1-scFv
 Peptide 2: VL-GR2-scFv



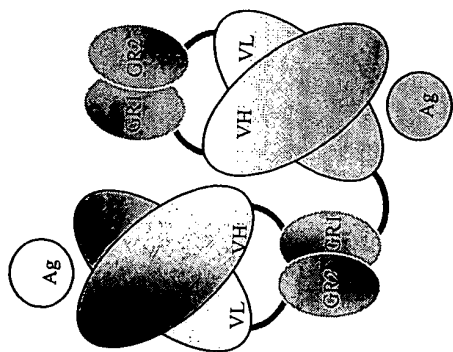
Tri-valent ccFv-scFv-dsFv
 Peptide 1: VL-GR2-scFv
 Peptide 2: VH-GR1-VH-s
 peptide 3: VL-s



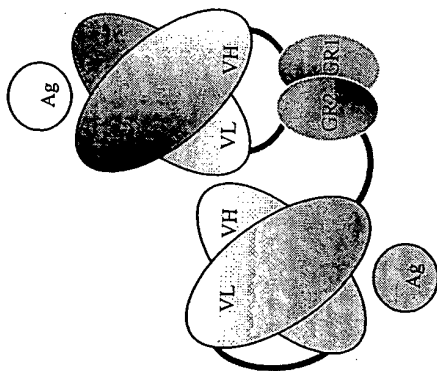
Tri-valent ccFv-(dsFv)2
 Peptide 1: VL-GR2-VL-s
 Peptide 2: VH-GR1-VL-s
 peptide 3: VH-s

Fig. 14

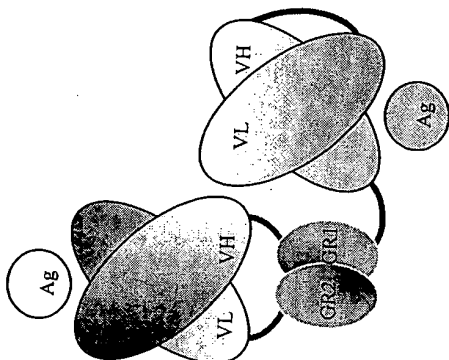
Bi-specific antibody (1)



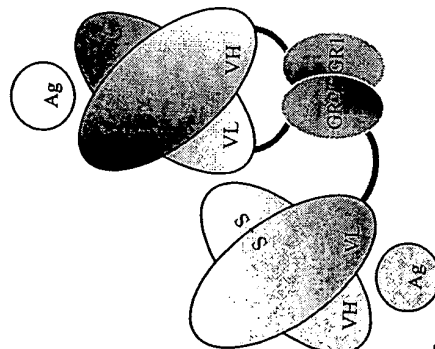
Bi-specific ccFv-ccFv'
 Peptide 1: VH-GR1-VL'-GR2
 Peptide 2: VL-GR2
 Peptide 3: VH'-GR1



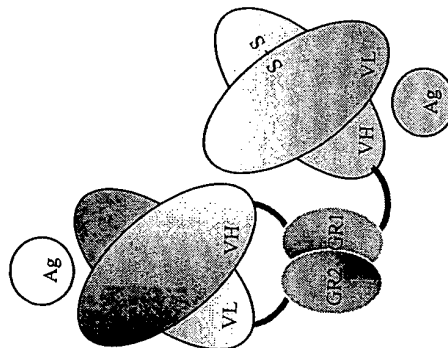
Bi-specific ccFv-scFv'
 Peptide 1: VL-GR2-scFv'
 Peptide 2: VH-GR1



Bi-specific ccFv-scFv'
 Peptide 1: VH-GR1-scFv'
 Peptide 2: VL-GR2



Bi-specific ccFv-dsFv'
 Peptide 1: VL-GR2-VL'-s
 Peptide 2: VH-GR1
 Peptide 3: VH'-s



Bi-specific ccFv-dsFv'
 Peptide 1: VH-GR1-VH'-s
 Peptide 2: VL-GR2
 Peptide 3: VL'-s

Fig. 15

Bi-specific antibody (2)

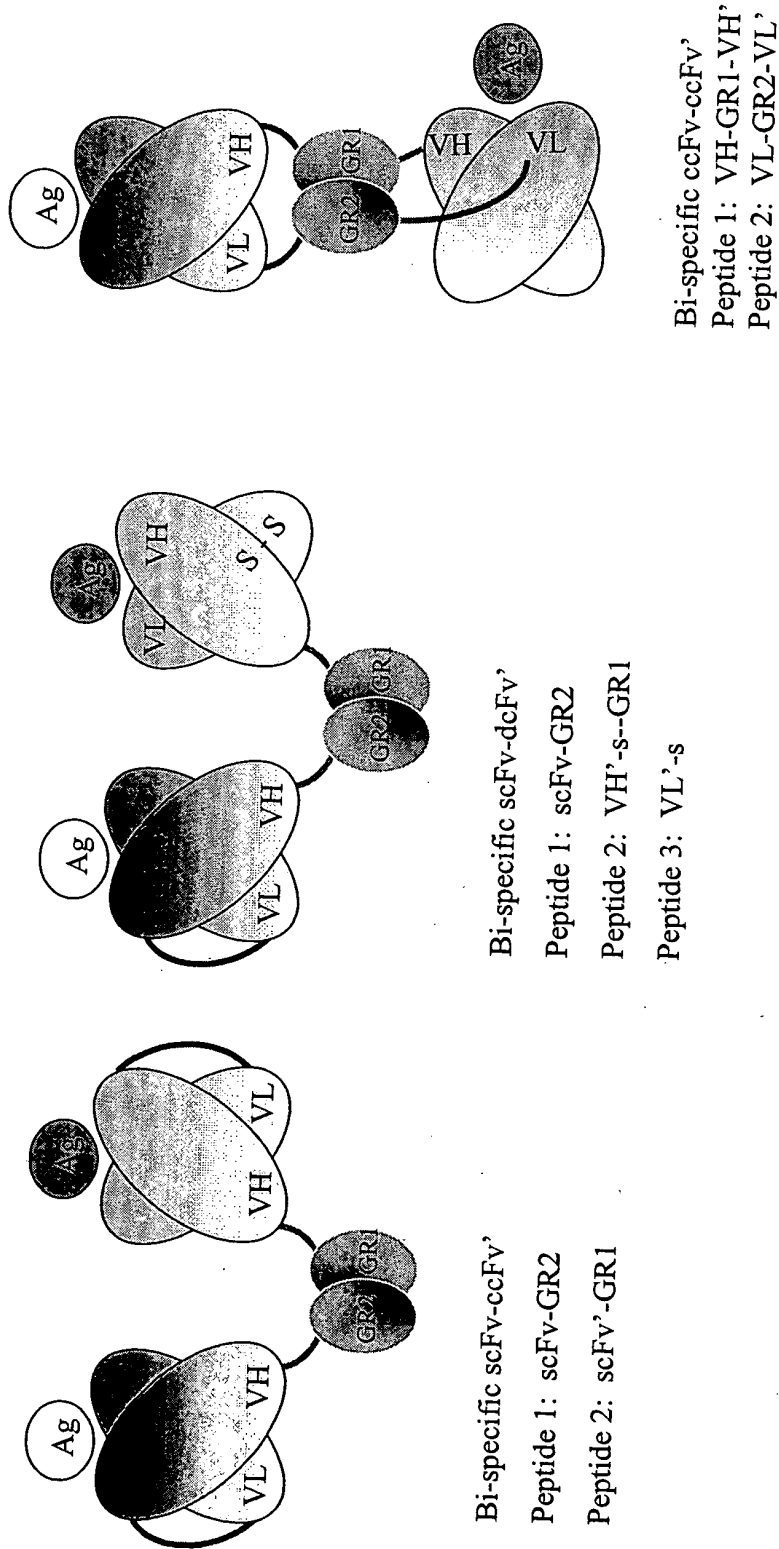
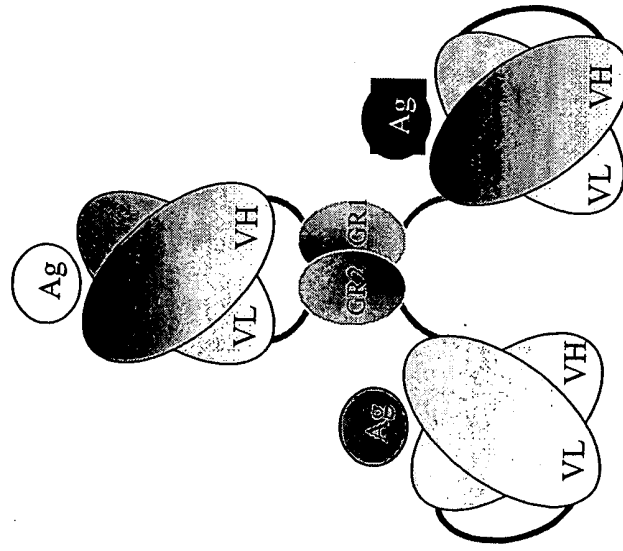
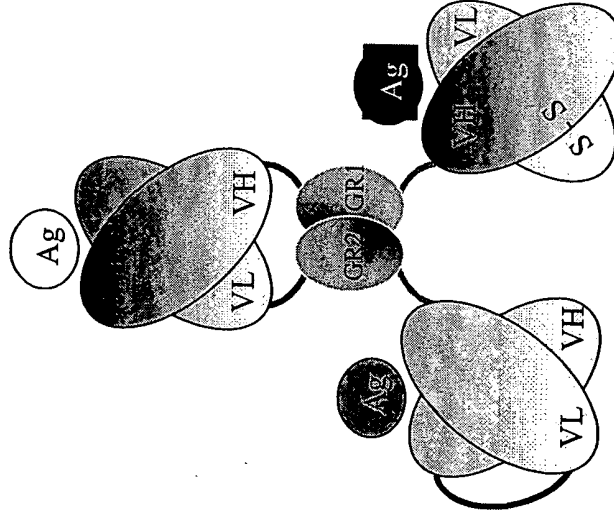


Fig. 16

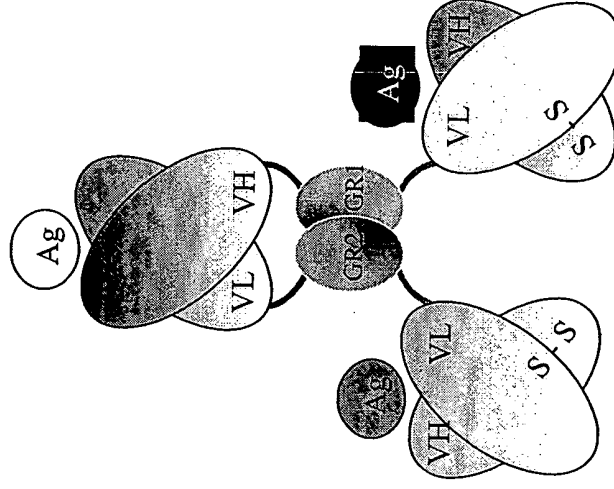
Tri-specific antibody



Tri-specific ccFv-scFv'-scFv*
 Peptide 1: VH-GR1-scFv'
 Peptide 2: VL-GR2-scFv*



Tri-specific ccFv-scFv'-dsFv*
 Peptide 1: VL-GR2-scFv'
 Peptide 2: VH-GR1-VH*-s
 peptide 3: VL*-s



Tri-specific ccFv-dsFv'-dsFv*
 Peptide 1: VL-GR2-VL'-s
 Peptide 2: VH-GR1-VL*-s
 peptide 3: VH'-s
 peptide 4: VH*-s

Fig. 17

107080-447266

Single-chain ccFv antibody

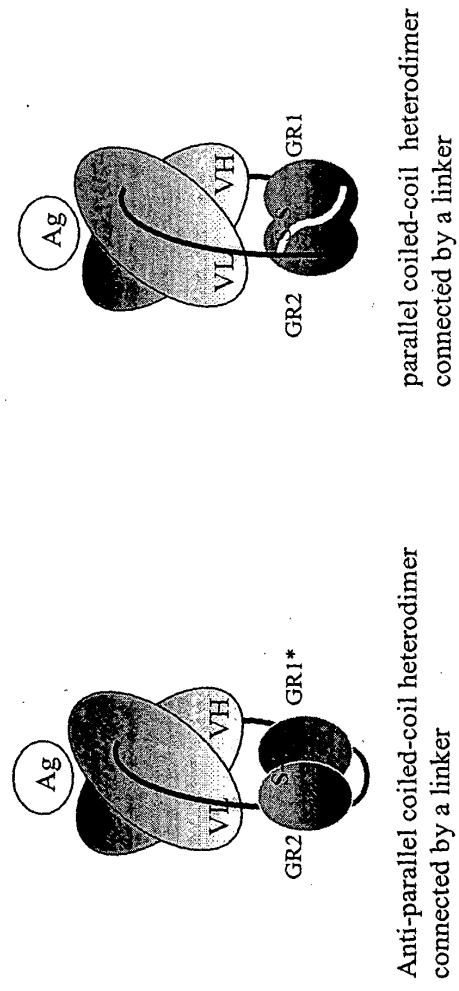


Fig. 18

ccFv display and its use in antibody library construction

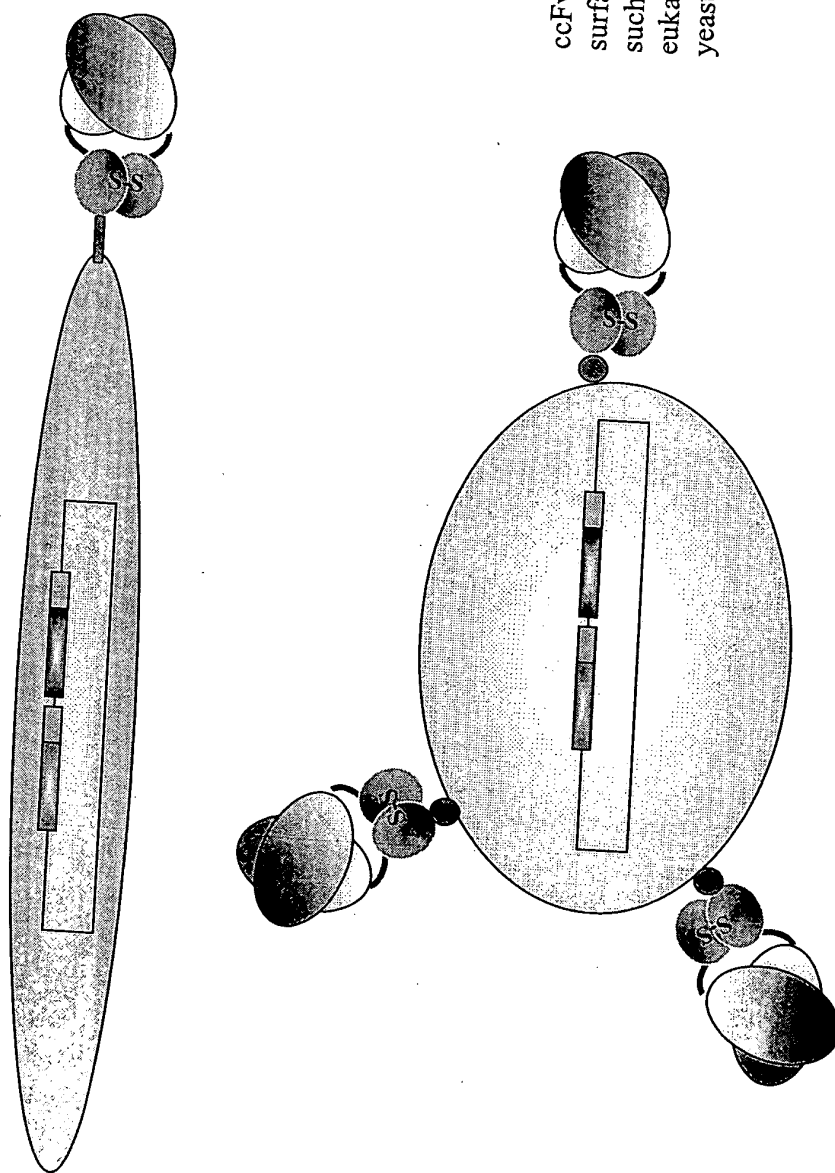


Fig. 19